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Research Progress on Molecular Mechanisms of Tumor Budding in Colorectal Cancer

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Abstract: Tumor buds are usually defined as isolated single cancer cells or clusters of up to four cancer cells located at the front of invasive tumors which play an important role in the clinical pathological study of colorectal cancer. The prognostic value of tumor budding in colorectal cancer has been supported by a large amount of evidence. However, its molecular mechanism remains unclear, and it is also a research hotspot now. This paper reviews the latest research progress on the molecular mechanisms of tumor budding in colorectal cancer.

Keywords: Tumor budding; Colorectal cancer; Molecular mechanisms

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1. Introduction

Colorectal cancer (CRC) is a common malignant epithelial tumor originated from the large intestine. Most of the cases are characterized by adenocarcinoma, which is a kind of tumor with gland structure characteristics [1]. Although surgical and systematic therapies have been effective for treating colorectal cancer in the past few decades, according to the latest statistics, the incidence rate and mortality rate of colorectal cancer is still the third and second in the world, respectively [2]. Improving the prognosis of colorectal cancer patients and influencing factors is an important method to reduce mortality.

TNM stage is always the most reliable prognostic factor of colorectal cancer defined by International Union Against Cancer (UICC) and American Joint Committee on Cancer (AJCC). The higher TNM stage, the worse prognosis. However, TNM staging does not consider other characteristics that allow risk stratification [3]. One of the prognostic factors is tumor budding (TB). Now, it is considered that TB is the first step of metastasis, because budding cells are thought to migrate through extracellular matrix, invade lymphatic vascular structure, and form metastatic deposits in distant lymph nodes [4]. TB is positively correlated with TNM staging of colorectal cancer [5]. Recently, as an additional prognostic factor, TB has been included in the eighth edition of AJCC colorectal cancer staging manual. UICC also recognizes that tumor budding is an independent prognostic factor of colorectal cancer.

There is a consensus that high TB is associated with poor prognosis. However, the potential molecular biological mechanism of TB is still unclear. This article reviews the latest research on molecular mechanism of TB in colorectal cancer in recent five years.

2. Laminin-5γ2 and integrin β activation of yes-associated protein 1 by promoting TB of colorectal cancer

Laminin (LN) is produced by the α， β and γ large heterotrimers composed of subunits that have different tissue specificity and developmental regulation patterns of expression [6]. Laminin is the receptor of integrins, and the subtypes of laminin, especially LN1 or LN5, are integrins β1 [7]. Integrin β1 is LN-5γ2. Downstream effectors of epithelial-mesenchymal transition (EMT) signal transduction [8]. EMT is a multi-step dynamic cellular phenomenon, in which epithelial cells lose intercellular adhesion and obtain the typical migration and invasion characteristics of mesenchymal cells [9].

The results showed that integrin β1 can promote TB, and its ligand LN5 can significantly promote tumor budding in a dose-dependent manner, which indicates that the promoting effect of LN5 on TB is dependent on integrin β1 [10]. The results showed that LN5 could promote TB and adhesion β. The germination promoting process of LN1 is dependent on LN-5γ2 subunit. For integrin β, the TB was inhibited and integrin β was found. The expression of integrin-1 was positively correlated with the ability of TB. Overexpression of integrin-1 significantly increased TB and decreased expression of integrin β. The TB was also significantly decreased after the expression of IL-1 [10].

Integrin β1 and LN-5γ by interaction with yes-associated protein (Yap) promotes TB by activating Yap translocation. LN-5γ2 and integrin β interaction between yap and transcriptional co-activator with PDZ-binding motif (TAZ) may activate the nuclear translocation of Yap/TAZ, a key downstream effector of Hippo-Yap pathway, and induce the spatial EMT characteristics of tumor cells. Hippo pathway is closely related to organ size control and tumorigenesis, and Yap/TAZ activity is the transducer of Hippo pathway. In addition, FAK-Src-PI3K was stimulated by fibronectin to regulate the Hippo pathway and Yap/TAZ subcellular localization in a dependent manner. The binding of integrin receptor with ECM can promote the autophosphorylation of FAK on y397 residue, regulate Hippo signal and activate Hippo component Yap to promote the invasion of adhesive plaque and tumor cells [11].

LN-5γ2 treatment and integrin β overexpression of polypyrimidine tract-binding protein 1 (PTB1) promoted TB in CRC cells, especially when the expression of both proteins were up-regulated. When Yap decreased, the above effects could be eliminated.

3. Relationship between neuronal Wiskott–Aldrich syndrome protein, LIM and SH3 protein 1 expression, and TB

LIM and SH3 protein 1 (LASP1) was initially identified from a gene library of breast cancer patients with axillary lymph node metastasis [12]. Now, it has been proven that LASP1 is a metastasis-related protein in CRC. Mediated EMT, invasive phenotype of cancer cells and progression of CRC are necessary [13,14]. LASP1 gene is located on chromosome 17q11-q21.3, and encodes a 261-amino-acid protein. It contains a LIM (lin1, Isl-1 and mec-3) domain at the N-terminal and a SRC homologous domain 3 (SH3) at the C-terminal. Therefore, most of the biological functions of LASP1 may be achieved through the protein-protein interaction between the two functional domains.

Neuronal Wiskott–Aldrich syndrome protein (N-WASP) is an important member of Wiskott-Aldrich protein family. N-WASP, which has an additional V domain, is widely distributed in human body. The expression of N-WASP was detected in CRC cells and overexpressed in most CRC tissues. The results showed that LASP1 was mainly expressed in the cytoplasm of CRC cells, while N-WASP was found in both cytoplasm and nucleus [15].

N-WASP is an important regulatory protein of actin cytoskeleton, which can affect the mobility, migration and invasion of cells by affecting the polymerization of actin [16]. Actin polymerization can change the cytoskeleton of actin and plays an important role in directional cell movement and migration. The full-length LASP1 and its SH3 domain can stimulate the interaction between N-WASP and actin-

related protein 3 (ARP3), indicating the potential role of LASP1 in N-WASP-mediated actin polymerization.

High expression of N-WASP was usually accompanied by high expression of Lasp1 and ARP3 ($P < 0.01$). The high expression of N-WASP is related to the increase of tumor seeding and the deterioration of invasion. Tumor budding is considered a marker of invasion and metastasis, which is closely related to the recurrence and poor prognosis of CRC [17]. The number of TB was significantly increased in CRC tissues with high N-WASP expression. N-WASP can stimulate the migration and invasion of CRC cells in vitro, and increase the formation of subcutaneous tumor, mesenteric implanted tumor and liver metastatic tumor. In CRC tissues with high N-WASP expression, the number of TB increased sharply ($P = 0.0315$). High N-WASP expression is positively correlated with tumor budding, which may be a predictor of poor prognosis of colorectal cancer [15].

By exploring the proteins interacting with lasp1, we can identify the new mechanism of LASP1-mediated CRC metastasis, and identify N-WASP as a potential therapeutic target for CRC.

4. S100A10 may be involved in the tumorigenesis of CRC

S100 protein family is composed of 21 members, belonging to the calcium binding protein superfamily. The protein family can show cell-specific expression and play multiple functions in cell formation, such as proliferation, differentiation and invasion. In addition, the change of S100 protein expression or function is a key step in the occurrence and development of cancer [18].

S100A10, a member of S100 protein family, is expressed in various cells, including cancer cells. It can form heterologous complexes with annexin A2 (ABX A2), then translocate from cytoplasm to cell membrane, and play a variety of roles in cell dynamics (including plasminogen activation) [19,20]. A recent study showed that S100A10 is involved in EMT [21]. S100A10 is an activator of heterologous complex, which can promote cell invasion. S100A10 is involved in the remodeling of actin and extracellular matrix (ECM), which is also related to EMT.

Regarding the relationship between S100A10 and TB of CRC, S100A10 and ABX A2 are not only related to poor differentiation, but also related to the budding of a special type of cancer cells, namely polyploid giant cancer cells (PGCC) [22]. PGCC is an established cancer cell line, which is induced by cobalt chloride or paclitaxel and develops in colorectal and other organs [22,23]. There is a close relationship between PGCC, TB, EMT and tumor differentiation [23]. It is reported that S100A10 and ABX A2 are highly expressed in PGCC and sprout [23].

However, the specific mechanism of S100A10 overexpression in TB remains unclear. However, in poorly differentiated cancer cells, the function of S100A10-ABX A2 heterocomplex is irregular, while another function (such as promoting cell migration or invasion [20,21,24,25]) is dominant. Tristante et al. reported that ABX A2 in CRC tumor bud has strong membrane immunity [26].

In the latest reported cases, immunohistochemical staining of S100A10 and ABX A2 showed diffuse positive results in TB and positive results in S100A10 membrane [27]. S100A10 immunoreactivity was observed in tumor cells protruding into the stroma. In contrast, even in tumor glands with TB, tumor cells with smooth boundaries around the stroma showed cytoplasmic granules expression in addition to reaction on the lumen surface. The immunoreactivity of ABX A2 was almost the same as that of S100A10. In addition, S100A10 and ABX A2 were positive on the surface of some tumors. In the main tumor components without TB or plasmacytoid dendritic cells (PDC), no positive membrane expression of S100A10 and ABX A2 was observed except for the reaction on the lumen surface.

Therefore, S100A10 may be related to TB in CRC during carcinogenesis. It is predicted that S100A10 can be added into TB, which is of guiding significance for the detection of carcinogenic process.

5. LGR5 RNA in situ hybridization in CRC with TB

Generally, cancer stem cells (CSCs) are considered to have the potential to form tumors and develop into cancer, especially with the metastasis of cancer, they are the source of new cancer [28]. So, in recent years, the importance of CSCs in cancer has been emphasized.

Leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5) has been identified as the most promising stem cell marker in CRC by pedigree tracing method and is considered the tumor stem cell marker in CRC [29,30]. Before tumor cell invasion, TB is at the forefront of tumor cell attack and host defense; therefore, TB provides important prognostic information [31,32]. It is found that the expression of LGR5 in TB is highly relevant.

The results showed that the expression of LGR5 was prominent in the glandular formation region. In LGR5-positive group, the incidence of tumor infiltrating lymphocytes (TIL) in budding area was lower than that in LGR5-negative group ($P = 0.0407$) [33]. The histological score of LGR5-positive group was lower than that of LGR5-negative group ($P = 0.0436$). However, there was no significant correlation between TB and LGR5 expression. Although LGR5 expression is not directly involved in TB, the correlation between LGRr5 expression and TIL can indicate that low level of TIL is involved in poor prognosis, and the correlation between high LGR5 expression and low TIL can indicate poor prognosis [34].

Studies have shown that high LGR5 expression in TB is associated with low TIL, and it is more common in cancer with low histological grade, which has been proven to be related to poor prognosis [33]. Therefore, LGR5 expression in TB may have important significance for CSC targeted therapy and has more reference significance for new molecular targeted therapy of CRC.

6. Human chorionic gonadotropin in CRC: The relationship between tumor budding and metastasis

Human chorionic gonadotropin (hCG) is a glycoprotein hormone that consists of two polypeptide subunits (α and β) [35]. In the absence free beta hCG, hCG is produced in ectopic condition. These tumor cells secrete beta hCG, which plays an autocrine role before invasion. It was found that tumors secreting beta hCG are more aggressive and resistant to radiation and tend to metastasize [36].

Tumor budding cells show a signal similar to EMT and activate transforming growth factor beta (TGF-β) and Wnt signal pathways [37]. Beta hCG can regulate the EMT in CRC. After beta hCG and TGF-β bind to their receptors, downstream cascade reactions are activated, leading to changes of ARP transcription. This can lead to morphological changes of tumor cells, thus acquiring mesenchymal phenotype and forming tumor buds. Beta hCG-induced overexpression of EMT-related proteins, such as E-cadherin, SNAIL, TWIST and phosphorylated Smad2, in CRC cell lines leads to mesenchymal phenotype and higher malignant potential compared with the control group. These changes result from the activation of TGF-β signaling pathways and the inhibitors of TGF-β receptor can effectively reverse these changes. Growing evidence shows that beta hCG β signal transduction acts through the TGF-β signaling pathway [38].

In an immunohistochemistry experiment, 13 out of 80 CRC patients (16.3%) were tested positive for beta hCG. There were more tumor buds in positive beta hCG cases ($P < 0.01$), and TB was related to beta hCG; the study also showed that there was a significant correlation between positive and negative results ($P<0.01$). Compared with all other groups, there were both tumor seeding and secretion of beta hCG, and the prognosis was the worst in the prognosis group ($P<0.01$). In a word, tumor seeding and beta hCG expression of EMT is closely related to CRC, and they are independent prognostic factors of CRC [39].

By activating TGF-β receptor, beta hCG induces the transformation of endometrial epithelial cells, and this corroborates the significance of TGF-β signaling in CRC. The expression of beta hCG in TB is a potential therapeutic target. In metastatic CRC, tumor seeding is associated with resistance to epidermal growth factor receptor antagonists [40]. For beta hCG-positive seeded cells, either vaccine or recombinant antibody may be a treatment option for advanced CRC.

The expression of TB and beta hCG are closely related and is common in CRC; therefore, patients with these two characteristics generally have poor prognosis [39]. They are also closely related to the occurrence and development of CRC and can be used as molecular targets for the treatment of CRC.

7. Conclusion

The prognostic value of TB in CRC patients is gaining clarity. It is important to note that TB is just a phenomenon, and the molecular mechanism underlying the phenomenon still remains to be elucidated. The existing literature shows that a variety of gene pathways and protein molecules are involved. It is of utmost important that the molecular changes of TB in CRC can be accurately identified. We should focus on patients with advanced CRC to whom surgical treatment is not recommended. We can use molecular means to detect gene changes in biopsy specimens to evaluate the prognosis. Targeted research on molecular mechanisms of TB may provide new ideas, specifically in targeting the expression of TB-related molecules, and modifying personalized treatment plan, so as to delay the progression of CRC, reduce the mortality, and further improve the prognosis of patients.

Disclosure statement

The authors declare that there is no conflict of interest.

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